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(54) Title: FEED SUPPLEMENT COMPOSITION

(57) Abstract

This invention relates to a feed supplement composition comprising one or more free fatty acids containing 6–10 carbon atoms or salts of such fatty acids, or mixtures of the aforementioned compounds. As a salt, preferably use is made of a $NH_4^+Na^+$, K^+ or Ca^{2+} salt. The present invention also relates to a feed composition comprising 10–30 percent by weight with respect to the weight of the total composition of the above described feed supplement composition.

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Feed supplement composition.

The present invention relates to a feed supplement composition as described in the preamble of the first claim.

In view of the economical interest of modern pig husbandry systems to increase productivity and maintain profitability, it has become general practice to increase the growth rate by subjecting piglets to an early weaning at an age of approximately 3 to 4 weeks. This early weaning however burdens the piglet with a lot of adverse stresses, mainly of nutritional origin. The adverse stresses are often accompanied by a more or less severe decrease in feed intake and energy deficiency and thus involve mobilization of body reserves by the piglet. Maldigestion and malabsorption may further aggravate the situation and result in digestive upsets due to bacterial overgrowth and/or viral infections of the intestines.

There is a general belief that the digestive pathology of early weaned pigs is mainly caused by Gram negative bacteria, in particular *Escherichia coli spp.* and *Salmonella spp.*, which are often present in the intestine of the piglet as such or may enter the gastro-intestinal tract through the feed. To overcome the problem of digestive pathology, which often involves a severe weight loss and an increased mortality amongst the piglets, it became common practice to supplement the piglet feed with low doses of pharmaceutical antimicrobial substances (for example antibiotics) or therapeutic doses of antibiotics, designated as "antibiotics" further on. The addition of antibiotics to the feed has further been found to result in a promotion of the growth of the piglets. Nowadays however, there is a growing concern on the addition of antibiotics to the

feed. There is a fear for the risk of the emergence of last-resort antibiotics used in human medicine, of the development of a resistance towards the antibiotics, which would involve a need to increase their dosing or to develop new, stronger antibiotics. There is also a fear that a resistance may emerge amongst living beings after consuming piglets that have been treated with those antibiotics. The present concern of environmental disturbance by chemicals and the fact that most of those antibiotics have already been banned in the EU in the near future, justify the need for alternatives. Moreover, the problems outlined above are not exclusively limited to piglets but may exist with other animal species and animals of other age groups.

There is thus a need to find a new feed composition as a substitute for the known and in the mean time banned antibiotics to overcome digestive pathology exhibited by cattle. In particular there is a need to find a substitute feed composition to overcome digestive pathology exhibited by poultry and young piglets after weaning.

It has now surprisingly been found that by supplementing the feed with a feed supplement composition which comprised one or more fatty acids containing 6-10 carbon atoms, the development of bacteria in the digestive tract of the young animal can be controlled. Most probably this effect can be explained by the fact that by the presence of these fatty acids in the stomach of the animal a physiological environment is created that is capable of regulating and stabilizing the gastro-intestinal microflora. It has been found that fatty acids containing 6-10 carbon atoms are capable of killing the majority of the pathological bacteria already in the stomach, so that the transit of pathological doses of bacteria towards the intestines can be prohibited and the occurrence of gastro-intestinal disorder prevented.

Besides the capability of regulating and stabilizing the gastro-intestinal microflora, fatty acids containing 6-10 carbon atoms have unexpectedly been found capable of markedly

improving the growth rate of the animal. The observed growth promotion ability of fatty acids containing 6-10 carbon atoms appears to be comparable with the growth promotion effects obtainable with the known antibiotic growth promoters, without however showing the above described adverse side effects for the animal, the feed industry and the consumer. According to the inventors, the growth promoting effect of the fatty acids containing 6-10 carbon atoms can be explained by their particular absorption from the digestive tract into the portal blood circulation system, as a consequence of which they constitute an energy source that is readily available to the animal. Fatty acids containing 6-10 carbon atoms namely form the main energy source for the development of mucosa and epithelial cells.

In this respect, the antimicrobial effects of fatty acids and their soaps show have been known for a long time and have been reviewed by J.J. Kabara in "The pharmacological effects of lipids", J.J. Kabara Ed. 1978, pp. 1-14. In this review it is discussed that in homologous series of fatty acids, the bactericidal efficiency has been found to increase with increasing chain length. *Escherichia coli spp.* and *Shigella spp.* appear to be killed by moderate concentrations of saturated soaps of lauric acid containing 10 carbon atoms, stearyl fatty acid containing 16 carbon atoms. Fatty acids with a chain length of 10 to 12 carbon atoms appear to show optimal antimicrobial activity, whereas lower fatty acids with 4-10 carbon atoms appear to have no or little germicidal effect. The mechanism according to which the fatty acids exert their antimicrobial action is also discussed. The consensus of the opinion is that the antimicrobial effects of short-chain fatty acids which contain 6-8 carbon atoms must be due to the non-dissociated molecule, not the anion. The activity of the fatty acids has been found to be profoundly affected by the pH of the medium, since this determines the degree of dissociation of the acid. An increase of the pH from 6.5 to 7.5 increased the minimum inhibitory concentration of the short chain fatty acids containing 6-8 carbon

atoms, and decreased the minimum concentrations of the two medium chained fatty acids containing 8-10 carbon atoms (lauric, myristic acid).

There is however no teaching in Kabara that fatty acids containing 6-10 carbon atoms would be capable of controlling the bacterial environment in the gastro-intestinal tract, nor that they would be capable of improving the growth rate of young animals.

In the present invention, preferably use is made of a mixture of different fatty acids, the individual fatty acids containing a different number of carbon atoms. The inventors have found that such a mixture shows optimal antimicrobial properties. Also with such a mixture, the antimicrobial spectrum of the antibiotic growth promoters used in today's intensive animal production can be mimicked approximately completely. This is surprising.

The feed supplement composition of the present invention preferably comprises a mixture of fatty acids, which contain 6-8 carbon atoms. The inventors have found that those fatty acids unexpectedly show the best antimicrobial properties and the most important growth rate enhancing effect.

The fatty acids are preferably present in the supplement composition in an amount of 0.01-2 percent by weight with respect to the total weight of the feed supplement composition.

The feed supplement composition of the present invention preferably contains an ammonium, a sodium, potassium or calcium salt of one or more of the free fatty acids, or a mixture of one or more of these salts, to prevent the composition from spreading an unpleasant odor which could restrain the animal from consuming it. Through the addition of a calcium salt of one or more of the fatty acids, the addition of lime stone as a calcium source can at least partly be dispensed with. The sodium salt of the fatty acids has been found to be capable of increasing the resorption efficiency of the fatty acid by the digestive system. This allows either to lower the amount of fatty acid to be supplied

or to further improve both the control of the microbial environment in the gastro-intestinal tract and the growth rate.

The feed supplement composition of the present invention preferably comprises slowly fermentable sugars (called prebiotics), preferably in an amount of 0.5 to 1.5 percent by weight with respect to the total weight of the composition. Suitable examples of prebiotics include slowly fermentable sugars for example galactomannans, for example guar gum, β-glucanes, transoligosaccharides, fructo-oligosaccharides and galacto-oligo-saccharides, but β-glucanes and galactomannans are preferred. Prebiotics have been found capable of inhibiting the attachment sites of pathogenic bacteria in the small and large intestine and in that way of lowering the pathogenic bacterial population at the end of the small intestine. Simultaneously they have been found capable of enhancing the development of bacteria in support of the well functioning of the large intestine, by counteracting reflux of pathogenic bacteria into the small intestine to a large extent.

The fatty acids that can be used in the feed supplement of this invention include both fatty acids with an even and an odd number of carbon atoms, for example C6 (caproic acid, hexanoic acid), C7 (heptanoic acid), C8 (caprylic acid, octanoic acid), C9 (nonanoic or pelargonic acid) and C10 (capric acid, decanoic acid).

The mechanism according to which the fatty acids exert antimicrobial activity has been well documented in literature. The currently accepted theory is that the lipid microbial cell membrane is permeable for the undissociated fatty acid, as a consequence of which the fatty acid is capable of passing across the microbial cell membrane towards the more alkaline interior. Because of the higher intracellular alkalinity, the fatty acid is dissociated, thus involving a decrease of the intracellular pH below the survival level. The fatty acid thus in fact acts as a protonophore, which increases inward leak of H^+ and involves that efflux of H^+ is too slow to allow the intracellular pH to be increased again. The

physicochemical properties of the fatty acids which allow them to act as protonophores, may vary and depend on numerous parameters. Examples of such parameters are the chain length and pKa of the fatty acid, as well as the physicochemical environment, precipitations, the pH in the different locations in the gastro-intestinal tract and the chemical composition of the microbial envelope which determines the passage of the fatty acids through the membrane. In this respect, the better performance of the fatty acid containing 6 carbon atoms is attributed to the extreme permeability of the microbial cell membrane for this fatty acid. This is quite unexpected, since Kabara discloses that the lower fatty acids containing 4-10 carbon atoms show little germicidal activity.

With respect to the observed growth enhancing properties of the fatty acids which contain 6-10 carbon atoms, it has been found that once these fatty acids have entered the gastro-intestinal tract, they are rather fast resorbed therefrom towards the portal blood circulation system. The inventors are of the opinion that this must be attributed to the fact that in the stomach of the animal the resorption can amount to 20-25% as compared to resorption by the villi of the small intestine, without necessitating the formation of micelles with bile acids. This means in fact that the energy contained in the fatty acids can be released without necessitating to start a fat digestion mechanism. Because the fatty acids containing 6-10 carbon atoms can be transported directly from the villi through the portal blood circulation system, towards the liver where they are oxidized, they constitute a readily available energy source, without being stack in adipose tissue.

The feed supplement composition of the present invention is preferably used as a supplement for the feed of early weaned piglets, but is not limited thereto. In fact, the feed supplement composition of this invention is also suitable for use with other animals for example poultry or other types of animals, as well as other age categories of animals. The feed supplement composition of this invention can for

example also be administered to sows, shortly before weaning of the piglets. In that way the fatty acids are administered to the piglets in an indirect manner and allow to prevent the development of pathogenic microorganisms in the stomach already before weaning. By administering the feed supplement composition of this invention to poultry or pigs, coccidioses which may involve *Closridium spp.* and necrotic enteritis, can be treated.

The invention also relates to a feed composition comprising the above described feed supplement composition, preferably in an amount of 10-30 percent by weight with respect to the total weight of the feed composition. Administration of such an amount allows to achieve an optimum growth performance .

The invention is further illustrated in the following examples.

Example 1: Feed composition.

A mixture according to this invention was prepared which contained approximately 40 parts by weight of barley, 14 parts by weight of wheat, 10 parts by weight of maize products, 11 parts by weight of Soya products and 20 parts by weight of a feed supplement composition containing 0.8 parts by weight of fatty acids with 8-10 carbon atoms.

A control feed was prepared which contained the same components as the above described mixture, with the exception that the control feed did not contain fatty acids.

Example 2: In vivo test with early weaned pigs.

A group of 10 pigs have been weaned after a period of 21 days. All pigs had free access to water and feed. A first control group (group 1) was fed with the control feed. 10^8 pathogenic bacteria (E. coli K88) were added per g of feed. A second group (group 2)

was fed with the feed composition of this invention as described in example 1. 10^8 pathogenic bacteria (E. coli K88) were added per g of feed. All animals were slaughtered 5 days after weaning. The number of bacteria per gram of stomach content was counted. The results are summarized in table 2.

Table 2. Amount of bacteria (in log of counted amount) per g of stomach content.

	Group 1	Group 2
Pig 1	3.9	6.5
Pig 2	2.5	<1
Pig 3	<1	<1
Pig 4	7.6	<1
Pig 5	7.8	<1

From table 2 it appears that by the addition of fatty acids which contain 8-10 carbon atoms bacteria are already killed in the stomach of the animals. In 80 % of the piglets fed with the feed supplement of this invention, hardly any bacteria could be found in the stomach, whereas with the control feed in only 20% of the cases bacteria could be killed already in the stomach.

It was further found that the feed intake of the control feed and the feed composition of this invention were approximately the same.

Example 3.

The experiment disclosed in example 2 was repeated. It has further been found that the group of pigs fed with the feed composition of this invention showed an improved growth performance of approximately 7.5 % than the control group. At an age of 55 days, the mean weight of the piglets was approximately 19 kg. Such piglets are

expected to reach the weight of 20 kg before day 60 of their life, which has been an objective that could not be reached for a long time. Also feed intake of the feed composition of this invention was slightly better than the usual feed.

Example 4.

Three samples of 100 ml of fermentation broth (Brain Heart Infusion) were equally inoculated with an overnight culture of *Escherichia coli K88* and further incubated at 37°C. The optical density at 600 nm (OD_{600nm}), which is proportional to the amount of colony formed was measured. As soon as an optical density at 600 nm (OD_{600nm}) of between 0.2 and 0.5 was obtained,

(1) nothing was added to the first sample,

(2) 12 ppm of colistine was added to the second sample and

(3) 1200 ppm of the sodium salt of a fatty acid mixture (BFC-dry) containing 50% of fatty acid with 8 carbon atoms and 50% of fatty acid with 10 carbon atoms, was added to the third sample.

The samples were further incubated at 37°C for 4 hours. The OD_{600nm} was measured evey hour. The samples were removed from the incubation after 4 hours, the pH was measured in order to register possible pH changes during incubation. The results are summarised in Table 3 given below.

Table 3.

	OD_{600nm}		
Hours after pH setting	Blank	Colistine	BFC-dry
2	0.478	0.420	1.259
3	0.491	0.449	1.276
4	0.558	0.455	1.262
Difference in OD_{600nm}	0.080	0.035	0.003

Growth %	100	44	4
final pH	4.04	3.93	4.28

As can be seen from figure 1 a linear relationship exists between OD_{600nm} and the amount of colony forming units (CFU). From the measured OD_{600nm}, the amount of colony forming units can be calculated. From table 3 it appears that the growth of *E. coli* is retarded for 56% by the addition of colistine and to 96% by the addition of BFC of this invention. As the pH in the three samples was approximately the same, the pronounced effect of the BFC of this invention.

CLAIMS.

1. Feed supplement composition comprising one or more free fatty acids containing 6 – 10 carbon atoms or salts of such fatty acids, or mixtures of the afore mentioned compounds.

2. Feed supplement composition as claimed in claim 1, characterized in that the feed supplement composition comprises a mixture of fatty acids containing 6-8 carbon atoms.

3. Feed supplement composition as claimed in any one of claims 1or 2, characterized in that as a salt use is made of a NH_4^+ Na^+, K^+ or Ca^{2+} salt.

4. Feed supplement composition as claimed in any one of claims 1-3, characterized in that it contains an amount of slowly fermentable sugars.

5. Feed supplement composition as claimed in claim 4, characterized in that it contains β-glucans.

6. Feed supplement composition as claimed in claim 4 or 5, characterized in that the slowly fermentable sugars are present in an amount of 0.5 to 1.5 percent by weight with respect to the total weight of the supplement composition.

7. Feed composition comprising 10-30 percent by weight with respect to the weight of the total composition of the feed supplement composition of any one of claims 1-6.

AMENDED CLAIMS

[received by the International Bureau on 06 June 2000 (06.06.00); original claims 1-7 replaced by new claims 1-11 (2 pages)]

1. Feed supplement composition comprising one or more free fatty acids containing 6 - 10 carbon atoms or salts of such fatty acids, or mixtures of the afore mentioned compounds.

2. Feed supplement composition as claimed in claim 1, characterized in that the feed supplement composition comprises a mixture of fatty acids containing 6 - 8 carbon atoms.

3. Feed supplement composition as claimed in claim 1, characterized in that the feed supplement composition comprises a mixture of fatty acids containing 8 - 10 carbon atoms.

4. Feed supplement composition as claimed in any of the claims 1, 2 or 3, characterized in that as a salt use is made of a NH_4^+, Na^+, K^+ or Ca^{2+} salt.

5. Anti-bacterial feed supplement composition as claimed in any of the claims 1-4.

6. Feed supplement composition as claimed in any of the claims 1-5, characterized in that it contains an amount of slowly fermentable sugars.

7. Feed supplement composition as claimed in claim 6, characterized in that it contains ß-glucan.

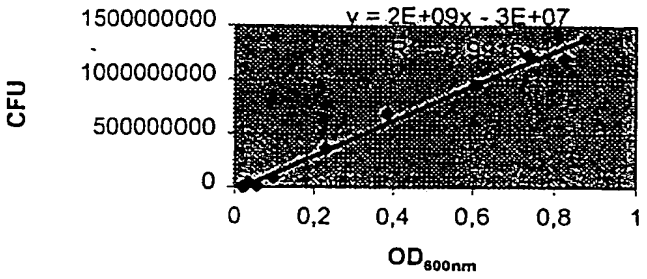
8. Feed supplement composition as claimed in claim 6 or 7, characterized in that the slowly fermentable sugars are present in an amount of 0.5 to 1.5 percent by weight with respect to the total weight of the supplement composition.

9. Feed supplement composition comprising 10-30 percent by weight with respect to the weight of the total composition of the feed supplement composition of any one of the claims 1-8.

10. Use of a feed supplement composition according to any of the claims 1-9 for use as an anti-bacterial agent.

11. Use according to claim 10 for the administration to young piglets after weaning.

Figure 1. Linear relation OD_{600nm} and CFU for *E. coli* K88



INTERNATIONAL SEARCH REPORT

Inte Jonal Application No
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A. CLASSIFICATION OF SUBJECT MATTER
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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	EP 0 519 458 A (KAO CORP) 23 December 1992 (1992-12-23) page 2, line 39-45 page 4, line 42 Examples claims 1,3	1,2,7

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
17 April 2000	02/05/2000

Name and mailing address of the ISA	Authorized officer
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Inte .onal Application No
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